JOURNAL OF AGRICULTURAL RESEARCH

VOL. 75 WASHINGTON, D. C., JULY 15, 1947 NO. 2

STORAGE OF COTTONSEED AND PEANUTS UNDER CONDITIONS WHICH MINIMIZE CHANGES IN CHEMICAL COMPOSITION [1]

By MACK F. STANSBURY, *assistant chemist*, and JOHN D. GUTHRIE, *senior chemist*, *Southern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

In the investigation of the chemical composition of cottonseed and peanuts, a method of storage which holds changes in chemical composition to a minimum is needed whenever samples are collected in considerable numbers and all cannot be worked on at the same time. Such a method of storage would also make it possible to keep at hand a collection of typical samples needed for investigative work extending over a considerable period of time. The chief object of the present investigation was to find a satisfactory method for storing cottonseed samples for at least a year without appreciable change in chemical composition. From a consideration of reports in the literature (*4*),[3] it would seem that the storage of peanuts does not present as difficult a problem as cottonseed storage. Nevertheless, a few samples of peanuts were included in the storage experiments.

Most seeds retain their viability best when kept dry and at low temperatures (*3*, *5*). Simpson (*10*, *11*) found this to be true for cottonseed. He also showed that little or no increase in free fatty acids occurred when cottonseed with a moisture content of less than 9 percent was stored for 2 years at 21° C. When cottonseed samples were stored at 1°, little or no increase in the free fatty acid content took place even at a moisture content of 14 percent. With the literature on seed viability and Simpson's experiments on cottonseed as a guide, it seemed likely that a satisfactory method for the storage of cottonseed would be to dry the samples to a moisture content of 8 percent or less and store at a temperature of 1° or below. However, the possibility that storage at a moisture content of 8 percent or less at room temperature (25°–28°) might prevent any appreciable change in chemical composition made it advisable to include this method of storage as well.

[1] Received for publication July 11, 1945.

[2] The authors wish to thank H. W. Barre and D. M. Simpson of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, for supplying the cottonseed, and B. B. Higgins, of the Georgia Agricultural Experiment Station, for supplying the peanuts. The authors are also indebted to Walter A. Pons, Jr., Vidabelle Orr, Richard H. Robinson, Alva Faust, and Dorothy H. Petty for aid in making some of the chemical analyses.

[3] Italic numbers in parentheses refer to Literature Cited, p. 61.

SOURCES OF SAMPLES

The cottonseed samples were obtained from the field deterioration studies of the Bureau of Plant Industry, Soils, and Agricultural Engineering. These samples included successive pickings from six varieties grown at six locations in the Cotton Belt in 1941 and seven varieties from a similar experiment in 1942. A few samples from the regional storage tests of the Bureau of Plant Industry, Soils, and Agricultural Engineering were used in some of the experiments.

Four samples of peanuts were obtained from the Georgia Agricultural Experiment Station.

METHOD OF STORAGE

Most of the cottonseed samples had a moisture content of between 7 and 8 percent at the beginning of the experiment. A few samples which contained more than 8.3 percent moisture were dried before storage by placing them in a closed 30° C. forced-draft circulatory oven containing a tray of anhydrous calcium chloride until the moisture content of the seeds reached about 7 percent.[4] The samples were mixed thoroughly, divided into four equal portions, and each portion was placed in a sealed container. One-gallon tin cans were used for the larger samples. These were sealed with paraffin. Mason jars were used for the smaller samples. One part of the sample was analyzed, and the other three parts were stored at room temperature, in commercial cold storage at 1°, and at −18° C.

The samples of unshelled peanuts were stored in closed, but unsealed, metal cans at room temperature, at 1°, and at −18° C.

METHODS OF ANALYSIS

Moisture, total nitrogen, oil, iodine number of the oil, and free fatty acid content of the oil were determined by the methods of the American Oil Chemists' Society (*1*). The method of Greenbank and Holm (*6*) was used for the determination of the peroxide number of the oil. Other methods are described in the following paragraphs.

FREE FATTY ACIDS IN THE OIL

Some of the cottonseed samples were too small for free fatty acid determinations by the method of the American Oil Chemists' Society. For these samples the following method, developed by Carroll L. Hoffpauir of this laboratory, was used:

Duplicate 2-gm. samples of kernels were obtained by removing the hulls. The kernels were cut fine or crimped and shaken with 100 ml. of Skellysolve F for 2 hours on a mechanical shaker. The extracted oil was obtained by evaporation on the steam bath of 50-ml. portions of the extract filtered into tared 100-ml. oil-extraction flasks. After the weight of the oil was determined, it was dissolved in 10 ml. of Skellysolve F and 25 ml. of redistilled 95-percent ethyl alcohol. About 5 drops of 0.05-percent *m*-cresol purple indicator were added,

[4] The possibility that drying in this way might affect the seeds adversely was checked by the determination of catalase activity before and after drying No appreciable change in catalase activity was found.

and the solution was titrated with 0.01 N alcoholic NaOH until a purple color appeared. A stream of CO_2-free air was bubbled through the solution during the titration. Blanks were run on the reagents and corrections made.

REDUCING SUGARS

Approximately 100 gm. of cottonseed were dried for 30 minutes at 100° to 105° C., cooled, and dehulled in a Bauer mill. The kernels were separated by screening and ground to a fine meal through the Bauer mill. Approximately 40 gm. of the ground kernels were weighed into extraction thimbles and extracted in a Soxhlet apparatus with Skellysolve F for 8 hours to remove most of the oil. The material was then extracted for 20 hours with 70-percent ethyl alcohol. The alcohol was removed from the extracts by heating on the steam bath with frequent addition of water, and the extracts were transferred to 500-ml. volumetric flasks, leaded, made to volume, and deleaded in the customary manner. Reducing sugars were determined on 50-ml. aliquots by precipitating cuprous oxide according to the Munson-Walker general method (*2, p. 500*), followed by use of the volumetric permanganate method of the Association of Official Agricultural Chemists (*2, p. 501–502*). Reducing sugars were calculated as glucose.

TOTAL SUGARS

Aliquots of the deleaded extracts prepared for the reducing-sugar determinations were used for the determination of total sugars, including raffinose. Aliquots of 100 ml. were placed in 250-ml. flasks and hydrolyzed by heating under reflux condensers for 2½ hours on the steam bath with 10 ml. of HCl (sp. gr. 1.125). This long hydrolysis was necessary to insure complete hydrolysis of raffinose. The solutions were cooled, neutralized with NaOH, and made to 250 ml. in volumetric flasks. Sugars were determined on 50-ml. aliquots in the same manner as for reducing sugars, and calculated as invert sugar. In some of the total sugar determinations, the 70-percent alcoholic extract of oil-free kernels prepared for protein solubility determinations was used.

RAFFINOSE

Aliquots of the deleaded extracts prepared for the reducing sugar determinations were used for the determination of raffinose in the presence of sucrose by the double enzymatic hydrolysis method of the Association of Official Agricultural Chemists (*2, p. 495*). Little, if any, sucrose was found in the samples of cottonseed kernels analyzed. The invertase was prepared from baker's yeast and the invertase-melibiase from brewer's yeast. Both solutions were concentrated by ultrafiltration (*2, p. 492*).

CATALASE

Approximately 8 gm. of cottonseed were obtained by mixing and reducing the entire sample. The seeds were split by hand, the kernels were removed, chopped fine, mixed thoroughly, and 1 gm. was transferred to a Waring blendor. The kernels were ground with 75 to 100 ml. of water, the sides of the blendor jar being washed down periodically with a little water. When a uniform suspension was obtained,

it was transferred to a volumetric flask and made to 200 ml. Catalase activity was determined immediately on 5-ml. aliquots, by using a modification by Davis of Appleman's apparatus described in Miller's Plant Physiology (*7*). Determinations were made at 25° C. with 5 ml. of extract, 5 ml. of pH 7.1 phosphate buffer, and 5 ml. of 3-percent H_2O_2 neutralized with $CaCO_3$. Volumes of oxygen were read at 1-, 2-, and 3-minute intervals, but only the 2-minute values will be reported here.

PROTEIN SOLUBILITY

The Skellysolve-F-extracted residues from the free fatty acid determinations were air-dried, ground to pass a 16-mesh screen, and extracted with diethyl ether for 36 hours in a Soxhlet extractor. The oil-free residue was ground to pass a 60-mesh screen, and hull fragments were removed by screening. The material was allowed to air-equilibrate and the relative protein solubility was determined by the method of Olcott and Fontaine (*8*). Results were calculated as percent of the total nitrogen peptized by 0.5 N NaCl solution.

EXPERIMENTAL RESULTS

Approximately 100 samples of cottonseed from the 1941 field deterioration studies of the Bureau of Plant Industry, Soils, and Agricultural Engineering were stored. The free fatty acid content of the oil was determined on all the samples at the time of storage and after storage. After the elimination of data on certain samples because of questionable accuracy of the original free fatty acid values, as indicated by their failure to correlate with germination values supplied with the samples, and the elimination of other samples too small to be included in all three storage conditions, the samples were grouped according to the original free fatty acid values and averages were calculated for each storage condition. The summary of these results is given in table 1. A small increase in the free fatty acid content occurred in the samples stored at room temperature (25°–28°), but no increase was observed in the samples stored at 1° and at −18° C. The apparent decrease in free fatty acids observed in some of the samples stored at −18° might not have been a real decrease, but possibly one arising from the difficulties inherent in the sampling of cottonseed. This conclusion received support from the data obtained in subsequent experiments, especially those reported in table 5.

TABLE 1.—*Free fatty acid content of the oil after storage of cottonseed in sealed containers at various temperatures*

Method	Range of free fatty acid content of oil	Samples	Average free fatty acid content of oil			
			Start of test	18 months 25°–28° C.	18 months 1° C.	18 months −18° C.
	Percent	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
A. O. C. S.	0.1 to 0.3	25	0.2	0.3	0.2	0.2
Do	.3 to 2.2	2	.5	.8	.5	.5
Do	2.2 to 30.0	3	19.3	22.8	18.5	16.7
Micro	.1 to .3	15	.2	.3	.2	.1
Do	.3 to 2.2	12	1.4	2.2	1.3	1.0
Do	2.2 to 12.0	6	7.2	7.9	5.9	

Five of the larger samples from the 1941 field deterioration studies were selected to cover the range of free fatty acid content of the entire group of samples. The results of analyses made for free fatty acid content of the oil, iodine number of the oil, peroxide number of the oil, total nitrogen content of the kernels, and catalase activity of the kernels are given in tables 2 and 3. The data show a small increase in the free fatty acid content of the oil in the samples stored for 18 months at room temperature, but no increase in the samples stored at 1° or −18° C. A small decrease in the iodine number of the oil may have occurred in the samples stored at room temperature, but otherwise no appreciable change in iodine number, peroxide number, total nitrogen content, or catalase activity took place under any of the storage conditions. It is of interest to note that the catalase activity of high free fatty acid cottonseed was considerably lower than that of low free fatty acid samples.

Six samples of low and high free fatty acid content from the 1941 crop were stored at room temperature for 24 months to ascertain whether or not any change in sugars would take place. The results are shown in table 4. A slight decrease in reducing sugars was observed for all samples. Although the data seem to show a small average increase in raffinose and a decrease in total sugars upon storage at room temperature, it is probable that such changes are due to errors inherent in sampling cottonseed and hydrolyzing sugar extracts and do not represent real changes. It is worth noting that the raffinose content of the kernels of low free fatty acid seeds was approximately 8 percent, as compared with 3 percent for high free fatty acid seeds.

Germination determinations on 10 selected samples stored for 18 months at room temperature, at 1°, and at −18° C. showed no appreciable change from the values found before the samples were shipped to this laboratory.

With the results on the 1941 storage experiments to serve as a guide, 20 samples were selected from the 1942 crop of the field deterioration studies mentioned above. These included seed samples which were high and low in free fatty acids and included 7 varieties of cottonseed. The 20 samples, with moisture content between 7.4 and 8.3 percent, were analyzed for total nitrogen and total oil content of the whole seed, and free fatty acid content of the oil. After storage for 14 months the samples were analyzed again. The results are given in table 5. There was no appreciable change in total nitrogen or oil content during storage, but a significant increase in the free fatty acid content of the oil occurred in the samples stored at room temperature. No significant change in free fatty acid content of the oil was observed in the samples stored at 1° or −18° C. for 14 months.

Seven samples of cottonseed selected for high and low free fatty acid content were analyzed for total sugars and relative protein solubility of oil-free kernels, stored for 14 months at the various temperatures, and analyzed again. The results are given in table 6. Apparently, a small increase in total sugars and in relative protein solubility took place during storage at all temperatures. It is likely, however, that this was due to some systematic error of sampling and analysis and that a significant increase did not occur. Especially is

TABLE 2.—*Analyses of the oil after storage of cottonseed at various temperatures*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content of oil				Iodine No. of oil (Wijs)				Peroxide No. of oil			
				At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.	At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.	At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.
				Percent	*Percent*	*Percent*	*Percent*								
A867	Farm Relief (strain 3).	Knoxville, Tenn.	Sept. 30, 1941	0.1	0.2	0.2	0.2	107	105	106	106	4	2	1	2
A874	Stoneville 37	Tifton, Ga	Aug. 21, 1941	.9	1.3	.9	.8	107	104	106	106	2	2	1	3
A924	do	Stoneville, Miss.	Sept. 26, 1941	.2	.2	.2	.2	111	109	110	110	2	2	2	4
AB202	Rowden 42A	Baton Rouge, La.	Sept. 10, 1941	10.9	13.9	11.0	10.1	106	104	106	106	1	1	1	2
AB203	Coker 100 (strain 3).	do	do	35.0	41.3	36.3	33.0	107	106	107	108	1	0	0	1

TABLE 3.— *Total nitrogen content and catalase activity of the kernels after storage of cottonseed at various temperatures*

Analysis No.	Total nitrogen (dry basis)				Catalase (ml. O_2 in 2 min.)			
	At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.	At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.
	Percent	*Percent*	*Percent*	*Percent*				
A867	6.49	6.58	6.65	6.68	12.9	13.4	13.3	12.1
A874	5.98	6.16	6.14	6.19	13.1	13.8	13.5	11.4
A924	5.02	4.98	5.12	5.14	10.5	11.0	11.0	9.9
AB202	6.35	6.49	6.46	6.45	4.1	5.1	5.7	5.3
AB203	6.83	6.87	6.80	6.80	3.4	4.0	3.9	3.0

this true of total sugars determinations, where acid hydrolysis of the sugar extracts probably gives variable results. Additional data for the protein solubility on 5 samples stored 14 months at room temperature are given in table 7. An increase in relative protein solubility was observed in only 1 of these samples. Very little change was found in the protein solubility of the extracted meal after it was stored for 14 months. This is in agreement with the results of Olcott and Fontaine (*9*). The pH values on the NaCl extracts of the meals were also observed to be relatively unchanged.

The results of an experiment in which four samples of unshelled peanuts were stored in closed, but unsealed, metal cans at room temperature, at 1°, and at −18° C., for 30 months, are shown in tables 8 and 9. A slight increase in the free fatty acid content was observed in room-temperature storage, but no increase occurred at 1° or at −18°. The increase in free fatty acids was very large for one of the samples that molded at room temperature. This sample had been analyzed at the end of 6 months' storage at room temperature and was then in good condition. The total nitrogen and oil content of the kernels and the iodine number of the oil remained relatively constant under all the storage conditions for all the samples except the one that molded at room temperature. Slight changes in the moisture content of the kernels in this experiment may have been due to the fact that the cans were not sealed. In contrast, none of the cottonseed samples, which were stored in cans sealed with paraffin or in mason jars, showed any appreciable change in the moisture content during storage at the various temperatures.

TABLE 4.—*Sugar content of the kernels after storage of cottonseed at room temperature*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content at start of test	Reducing sugars (dry basis)		Raffinose (dry basis)		Total sugars (dry basis)	
					At start of test	24 months, 25°–28° C.	At start of test	24 months, 25°–28° C.	At start of test	24 months, 25°–28° C.
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A880	Farm Relief (strain 3).	Tifton, Ga.	Aug. 21, 1941	0.3	0.3	0.2	7.6	7.6	7.8	7.0
A893	Coker 100 (strain 3).	Florence, S. C.	Sept. 4, 1941	.3	.4	.2	7.8	8.6	8.2	8.0
A932	do.	Stoneville, Miss.	Sept. 26, 1941	.2	.3	.2	7.8	7.2	8.4	7.4
A935	Farm Relief (strain 3).	do.	Sept. 12, 1941	.4	.3	.2	7.7	8.1	8.3	7.8
AB201	Stoneville 37.	Baton Rouge, La.	Sept. 10, 1941	30.6	.1	0	3.3	4.0	3.5	3.7
AB203	Coker 100 (strain 3).	do.	do.	35.0	.1	0	2.4	2.5	3.1	3.2

TABLE 5.—*Effect of storage of cottonseed at various temperatures on its chemical composition*

Analysis No.	Variety	Place grown	Date of picking	Total nitrogen (dry basis)				Oil (dry basis)				Free fatty acid content of oil			
				At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.	At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.	At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
AG189	Acola	Tifton, Ga	Sept. 2, 1942	3.71	3.71	3.72	3.77	23.1	22.4	21.6	21.7	0.9	1.4	1.1	0.8
AG192	Arkansas Green Lint.	do	do	3.41	3.38	3.43	3.48	21.6	21.0	21.1	9 20.3	.4	.5	.6	.6
AG197	Farm Relief (strain 3).	do	Aug. 19, 1942	3.45	3.28	3.22	3.44	15.6	16.1	15.6	15.9	.6	.9	.6	.5
AG201	Rowden 42A	do	Sept. 2, 1942	3.66	3.63	3.69	3.72	19.1	19.0	18.7	18.9	.4	.5	.5	.5
AG217	Arkansas Green Lint.	Knoxville, Tenn.	Nov. 16, 1942	3.18	3.22	2.91	3.13	24.7	23.4	23.2	23.3	.6	1.0	.8	.7
AG220	Coker 100 (strain 3).	do	Oct. 19, 1942	3.10	2.85	2.93	2.99	19.2	19.5	18.5	18.9	.2	.2	.3	.3
AG239	Stoneville 37	do	Nov. 16, 1942	3.14	3.15	3.02	3.09	22.1	21.8	21.3	21.3	.3	.8	.4	.3
AG241	Acala	Greenville, Tex.	Aug. 26, 1942	3.67	3.61	3.63	3.62	23.3	22.9	23.3	23.1	.3	.5	.4	.4
AG253	Farm Relief (strain 3).	do	Sept. 9, 1942	3.12	3.33	3.24	3.37	21.3	21.0	21.3	21.7	.2	.3	.3	.3
AG260	Seabrook (sea island).	do	Sept. 23, 1942	3.15	3.14	3.27	3.15	19.7	19.8	19.3	19.5	1.3	1.7	1.7	1.6
AG274	Coker 100 (strain 3).	Baton Rouge, La.	Sept. 25, 1942	3.35	3.37	3.33	3.43	18.1	17.7	17.8	16.8	2.0	3.0	2.3	2.2
AG278	Rowden 42A	do	Sept. 11, 1942	3.51	3.49	3.49	3.54	21.1	21.0	20.4	20.8	2.6	3.5	2.1	2.0
AG301	Stoneville 37	Stoneville, Miss.	Sept. 14, 1942	3.38	3.35	3.51	3.48	20.6	20.3	19.6	20.3	.2	.2	.3	.2
AG305	Acala	Florence, S. C.	Sept. 3, 1942	3.46	3.56	3.48	3.47	25.6	25.5	24.8	25.6	4.1	5.2	4.0	3.8
AG308	Arkansas Green Lint.	do	Aug. 20, 1942	3.02	2.97	2.82	2.79	23.7	24.0	23.8	24.2	3.8	4.8	3.7	3.1
AG313	Coker 100 (strain 3).	do	Sept. 3, 1942	2.88	2.89	2.80	2.83	20.9	20.4	20.7	20.4	1.9	2.3	1.8	1.6
AG318	Farm Relief (strain 3).	do	Sept. 17, 1942	3.38	3.21	3.21	3.34	22.2	23.0	21.5	22.6	1.7	2.8	1.9	1.8
AG321	Rowden 42A	do	Sept. 3, 1942	3.19	3.27	3.20	3.18	22.8	22.2	21.9	21.5	2.0	2.3	1.7	1.6
AG327	Stoneville 37	do	do	2.95	2.88	2.89	2.87	21.8	21.9	22.1	21.6	1.3	2.0	1.4	1.4
AG328	do	do	Sept. 17, 1942	3.01	3.03	2.90	2.87	20.8	20.9	20.5	20.7	.3	.5	.3	.3
Average				3.29	3.27	3.23	3.28	21.4	21.2	20.9	21.0	1.3	1.7	1.3	1.2

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TABLE 6.—*Total sugars content and relative protein solubility of the ether-extracted kernels after storage of cottonseed at various temperatures*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content at start	Total sugars (dry basis)				Relative protein solubility [1]			
					At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.	At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.
				Percent	*Percent*	*Percent*	*Percent*	*Percent*				
AG 253	Farm Relief (strain 3).	Greenville, Tex.	Sept. 9, 1942	0.2	9.8	14.2	14.0	14.0	79	79	80	81
AG 260	Seabrook (sea island).	do	Sept. 23, 1942	1.3	11.2	12.4	12.2	14.4	67	71	71	70
AG 305	Acala	Florence, S. C.	Sept. 3, 1942	4.1	9.1	12.6	13.5	13.7	64	79	78	77
AG 308	Arkansas Green Lint.	do	Aug. 20, 1942	3.8	11.1	12.9	14.3	14.8	69	75	76	69
AG 313	Coker 100 (strain 3).	do	Sept. 3, 1942	1.9	10.9	13.2	14.8	14.5	72	75	78	74
AG 321	Rowden 42A	do	do	2.0	10.7	13.4	14.3	14.6	76	78	79	79
AG 328	Stoneville 37	do	Sept. 17, 1942	.3	13.1	12.1	14.7	15.0	75	79	77	79

[1] Percentage of total nitrogen peptized by 0.5 N NaCl solution.

TABLE 7.—*Relative protein solubility of the ether-extracted kernels after storage of cottonseed and cottonseed meal at room temperature*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content at start of test	Relative protein solubility [1]		
					Start of test	Seed stored 14 months at 25°–28° C.	Meal stored 14 months at 25°–28° C.
				Percent			
AG 269	Arkansas Green Lint	Baton Rouge, La.	Aug. 28, 1942	6.5	76	74	74
AG 272	Coker 100 (strain 3)	do	do	21.0	66	65	63
AG 281	Stoneville 37	do	do	19.9	68	64	65
AG 316	Farm Relief (strain 3)	Florence, S. C.	Aug. 20, 1942	22.5	70	68	67
AG 324	Seabrook (sea island)	do	Sept. 3, 1942	3.3	57	74	55

[1] Percentage of total nitrogen peptized by 0.5 N NaCl solution.

TABLE 8.—*Analyses of the oil in the kernels after storage [1] of unshelled peanuts at various temperatures*

Analysis No.	Variety	Free fatty acid content of oil				Iodine No. of oil (Wijs)			
		At start of test	30 months, 25°–28° C.	30 months, 1° C.	30 months, −18° C.	At start of test	30 months, 25°–28° C.	30 months, 1° C.	30 months, −18° C.
		Percent	*Percent*	*Percent*	*Percent*				
AB 406	Tennessee Red	0.2	0.3	0.1	0.1	98	97	98	97
AB 407	Jumbo Virginia Runner.	.2	[2] 14.2	.1	.1	87	86	87	87
AB 408	Spanish (commercial strain).	.3	.6	.2	.2	95	95	95	95
AB 409	Spanish (Ga. Agr. Expt. Sta.).	.5	.5	.2	.5	96	96	96	95

[1] Stored in closed, but unsealed, metal cans.
[2] This sample molded.

TABLE 9.—*Analyses of the kernels of unshelled peanuts after storage [1] at various temperatures*

Analysis No.	Variety	Moisture				Total nitrogen (dry basis)				Oil (dry basis)			
		At start of test	30 months, 25°-28° C.	30 months, 1° C.	30 months, −18° C.	At start of test	30 months, 25°-28° C.	30 months, 1° C.	30 months, −18° C.	At start of test	30 months, 25°-28° C.	30 months, 1° C.	30 months, −18° C.
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
AB 406	Tennessee Red	4.7	5.8	6.2	5.9	5.74	5.69	5.77	5.83	48.2	48.8	48.6	48.7
AB 407	Jumbo Virginia Runner	4.9	[2] 14.8	6.4	6.0	5.04	[2] 6.04	5.02	5.11	50.3	[2] 47.3	50.3	50.7
AB 408	Spanish (commercial strain)	5.0	5.8	6.8	6.0	5.17	5.20	5.22	5.22	48.5	49.1	48.9	48.9
AB 409	Spanish (Ga. Agr. Expt. Sta.)	4.8	7.0	6.6	6.1	5.09	5.11	5.11	5.16	48.5	49.3	49.0	49.1

[1] Stored in closed, but unsealed, metal cans.
[2] This sample molded.

SUMMARY

Cottonseed samples may be stored for more than a year without appreciable change in total nitrogen, total oil, free fatty acid content, iodine, or peroxide number of the oil, or catalase activity of the kernels by drying to a moisture content of 8.3 percent or lower, placing in sealed containers, and storing at 1° C. or below. Under this type of storage at room temperature a small increase in the free fatty acid content of the oil occurs. Unshelled peanuts may be stored for more than 2 years in closed cans at 1° C. or below without appreciable change in the total nitrogen or oil content of the kernels, or in free fatty acid content or iodine number of the oil.

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NONPROTEIN AND CAROTENE AS AN INDEX OF PLANT ACTIVITY IN RANGE FORAGE[1]

By W. E. WATKINS

Nutrition chemist and associate in animal husbandry, New Mexico Agricultural Experiment Station

INTRODUCTION

The protein contained in plant tissues is combined in two general classifications of compounds; true proteins and nonprotein nitrogenous substances of simpler composition such as amides, amino acids, and their derivatives. These nonprotein nitrogen compounds, according to Maynard (*3*)[2] and Morrison (*4*), are abundant when plant growth is rapid but are less plentiful at maturity.

It is also generally agreed, as stated by Maynard (*3*), that all green parts of growing plants are rich in carotene, while mature plants contain much less. Negligible carotene values have been reported by Watkins (*6*) during the periods of dormancy. In experiments with rats Smith and Stanley (*5*) found the early cut blue grama grass to be twice as potent as the mature grass in vitamin A, and 100 times as potent as the November cuttings.

It is generally accepted that the percentages of nonprotein nitrogen and carotene in plants are higher during growth than during dormancy, but just how much higher has not been known. In particular, there has been very little information concerning the amount of nonprotein nitrogen present in some of the range grasses and browse plants during the various stages of growth and maturity. This paper reports the results of monthly and regular sampling of various range grasses and browse plants of southern New Mexico covering a period of 65 months, together with nonprotein nitrogen values for 11 of the principal species of grasses and 2 browse plants; carotene values are reported for 2 of the principal grasses.

EXPERIMENTAL PROCEDURE

The arrangement of the plots and the system of collection of the range forages have been reported in detail by Watkins (*7*). The grass was harvested to a height of 2½ or 3 inches from the ground depending upon the species. The January samples include all growth produced since the preceding January. Likewise the August samples include all growth produced since the previous August. The duration of this study was 65 months. One portion of the sample was taken immediately for carotene analysis while the other portion was placed in canvas sacks, weighed, and kept in a drying oven for 7 days at a

[1] Received for publication August 3, 1945.

[2] Italic numbers in parentheses refer to Literature Cited p. 69.

temperature of approximately 60° C. It was then placed in an unheated conditioning oven for another 7 days, after which it was pulverized in a Wiley mill and placed in glass-stoppered sample bottles for analysis. This plan was rigidly followed for all samples.

The total nitrogen and the albuminoid nitrogen were determined on the various forages by the methods of the Association of Official Agricultural Chemists (*2*). The nonprotein nitrogen values are the total nitrogen less the albuminoid nitrogen. These three different nitrogen systems were converted to protein by the use of factors. The total nitrogen was changed to crude protein by using the factor 6.25, and the albuminoid nitrogen was converted to true protein by using the same factor. The nonprotein nitrogen was converted to nonprotein by using the factor 4.7 as suggested by Armsby (*1*). Hereafter in this paper the data and discussion will be with the various proteins rather than with the various nitrogen systems.

The carotene was analyzed according to the method of the Association of Official Agricultural Chemists (*2*) with slight modifications (*6*). Briefly, the procedure consists of subjecting the pulverized sample to saponification in aldehyde-free alcholic potassium hydroxide and extracting with peroxide-free ethyl ether. The ether solution, after washing free from chlorophyllins, flavones, and alkali, is distilled under reduced pressure, the residue taken up in petroleum ether (boiling point 35°–60° C.), and the xanthophyll removed by washing with 92-percent methanol.

The most important range grasses of southern New Mexico, which were studied intensively and sampled monthly, are black grama (*Bouteloua eriopoda*) and mesa dropseed (*Sporobolus flexuosus*). These were harvested from plots on the experimental ranch of the New Mexico College of Agriculture and Mechanic Arts, which lies at an altitude of approximately 4,200 feet above sea level and has an average rainfall of 9.3 inches, of which slightly more than 50 percent usually occurs in July, August, and September. This is normally the season of comparatively rapid plant growth.

Vince-mesquite grass (*Panicum obtusum*), alkali sacaton (*Sporobolus airoides*), and side-oats grama (*Bouteloua curtipendula*) were harvested and sampled every 60 days, while saltgrass (*Distichlis stricta*), bush muhly (*Muhlenbergia porteri*), chamiza (*Atriplex canescens*), and sand sagebrush (*Artemisia filifolia*) were harvested and sampled every 90 days. These forages were collected from the experimental ranch.

Range forages were also collected from plots near Estancia in central New Mexico, where the altitude is approximately 6,140 feet above sea level. Average annual precipitation is about 13 inches. Maximum temperatures in this vicinity seldom exceed 98° or 99°, but minimum temperatures of 10° to 20° below zero are not uncommon. The forages were collected four times a year from this area and included blue grama (*Bouteloua gracilis*), slender wheatgrass (*Agropyron pauciflorum*), crested wheatgrass (*A. cristatum*), smooth bromegrass (*Bromus inermis*), saltgrass, alkali sacaton, and chamiza.

EXPERIMENTAL RESULTS

Two range grasses which were studied rather intensively were mesa dropseed and black grama. In addition to the monthly harvest, other

samples were secured at various times during the growing season. The data for these two grasses, which include average results for crude protein, nonprotein, and carotene over a period of 5½ years, are presented in table 1.

TABLE 1.—*Average monthly crude protein, nonprotein, and carotene values for mesa dropseed and black grama over a period of 65 months*

Time collected	Mesa dropseed				Black grama			
	Number of analyses	Crude protein	Nonprotein	Carotene	Number of analyses	Crude protein	Nonprotein	Carotene
		Percent	*Percent*	*Milligrams* [1]		*Percent*	*Percent*	*Milligrams* [1]
January	5	4.48	0.59	1.3	5	5.01	1.01	23.6
February	5	4.40	.53	1.2	5	4.85	1.10	13.3
March	5	3.86	.63	1.3	5	4.74	.93	13.3
April	5	3.82	.45	11.1	5	5.10	1.00	22.7
May	5	5.59	1.10	23.0	5	5.16	1.02	26.6
June	5	5.40	1.02	39.3	5	4.73	.95	40.1
July	5	6.93	1.06	61.2	5	7.05	1.17	44.7
August	6	8.34	1.66	70.6	6	6.85	1.51	61.3
September	6	10.07	2.23	60.1	6	8.73	1.78	85.6
October	6	7.74	1.35	30.9	6	7.13	1.43	60.7
November	6	5.94	.84	3.7	6	5.51	1.21	41.9
December	6	4.89	.64	1.1	6	5.35	1.10	23.1
30-day growth	11	12.48	2.61	----------	11	10.10	2.24	----------
15-day growth	13	13.04	2.53	----------	11	11.20	2.44	----------
Beginning of growth	5	11.57	2.05	----------	4	10.64	1.95	----------
End of growth	3	8.36	1.77	----------	4	6.73	1.53	----------

[1] Milligrams of carotene per kilogram of feed, dry basis.

The samples designated by "30-day growth" were from the series which were collected every 30 days during the growing period; those designated "15-day growth" were collected every 15 days during the growing period. These two series represented material from immature but actively growing grass. The "Beginning of growth" samples were taken once a year at the beginning of the growing season when the grass had attained a height of 5 or 6 inches. The samples collected at the end of growth were a separate series and were taken as soon as frost occurred.

A comparison of the nonprotein, crude protein, and carotene content of mesa dropseed will indicate at least the general pattern of growth habit of this plant. Crude protein values were highest during September, which shows this to be the period of most vigorous growth. If the carotene values are considered as an index of plant activity, the mesa dropseed was active from April to November. Usually the mesa dropseed grass dies virtually to the ground with the advent of freezing weather the last of October or the first of November. Mere traces of carotene were found in dormant mesa dropseed plants during December, January, February, and March. If the nonprotein values are studied during these months when the grass is dormant, it will be seen that they were rather low, averaging approximately 0.61 percent. Values of over 1.00 percent were obtained from May to October inclusive. Plants harvested during the period of most active growth had nonprotein values of from 2.05 to 2.61 percent; thus, high nonprotein values were directly related to vigorous growth. The nonprotein percentages of the grasses collected every 15 or 30 days during

growth are among the highest values obtained with plants other than browse and apparently represent active growth rather than mere activity.

Black grama grows on the mesas, hills, and dry open ground of large areas of range land in Texas, New Mexico, Arizona, and southern Utah. The upright stems of this grass remain green for a distance of 4 to 6 inches from their bases throughout the winter. A comparison of the crude protein of black grama with that of mesa dropseed in table 1 reveals higher values for the mesa dropseed during the growing season, but higher values for black grama during the so-called dormant season. Carotene is present in black grama throughout the year. During the usual months of dormancy there are significant amounts of carotene that may in many cases provide for the requirements of beef cattle during the gestation and lactation periods (*6* and *7*). These winter carotene values are accompanied by winter plant activity. All the average monthly nonprotein values of black grama were above or only slightly below 1.00 percent. The analyses of this grass revealed 11 instances of low nonprotein values. The average of these 11 values was 0.66 percent and may represent the nonprotein content of black grama in nearly complete dormancy. This quantity is very similar to that obtained for mesa dropseed during its dormant period. If this figure of 0.66 percent is subtracted from the average nonprotein of black grama for each month, the remaining nonprotein value seems to accompany certain plant activity. In black grama this plant activity continues throughout the year, although at a much reduced rate.

The carotene and nonprotein values for the mesa dropseed and black grama grasses presented in table 1 agree in showing plant activity. When the simple correlation was calculated between carotene and nonprotein, a coefficient of 0.625 percent was obtained for black grama and 0.48 percent for mesa dropseed (table 2). These correlations are highly significant and seem to establish the fact that nonprotein values as well as carotene may be used in estimating plant activity. Although there is a relation between these numerical values and plant activity, the proportion does not appear to be direct. The nonprotein and carotene data for each of the two grasses have been averaged by months and are presented in figure 1 for comparison.

TABLE 2.—*Correlation of nonprotein (x) and carotene (y) in black grama and mesa dropseed*

Grass	Number of comparisons	Sum of squares and products			Correlation coefficient
		Sx^2	Sxy	Sy^2	
Black grama	59	8.34	411.06	52,064.4	0.625*
Mesa dropseed	59	22.44	634.30	76,769.8	.48*

*Highly significant.

A number of other grasses and forage plants were studied during regular but less frequent intervals. It is obvious that with 60- or 90-day sampling, the data are necessarily incomplete. However, there are observations which are worthy of attention. In studying the

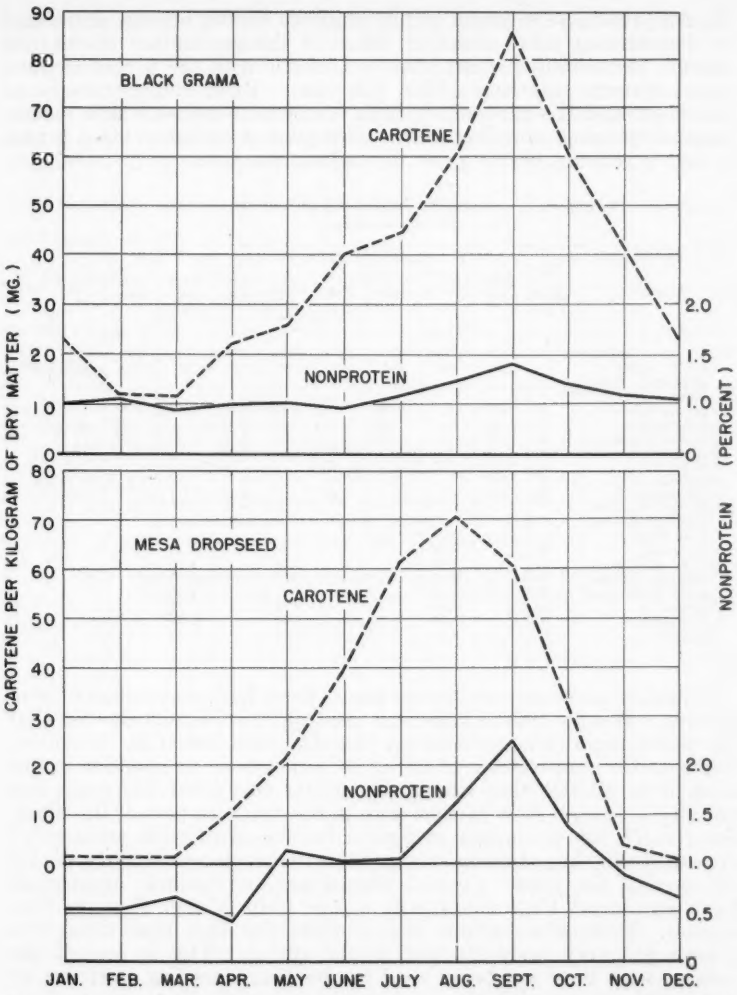

FIGURE 1.—Average carotene and nonprotein content of mesa dropseed and black grama covering 59 months.

nonprotein values for various grasses and browse plants, which are presented in table 3, it will be assumed, as stated earlier in this paper, that a nonprotein value of over 0.66 percent represents an increment of plant activity. The value of 0.66 percent seems satisfactory for black grama, but may be slightly high for the dormant period of some of the other grasses as is indicated by their February and March values. Since this seems to be true with this limited amount of data,

the nonprotein percentages of 0.66 or above readily become significant in determining plant activity. Most of the grasses are above 0.66 percent in nonprotein from May to October with the period of most active growth occurring within this time. Bush muhly grass is an exception and has moderately high nonprotein values which remain constant for each sampling time. This grass is similar to black grama in that it remains partly green throughout the year.

TABLE 3.—*Percentage of nonprotein in various grasses and browse collected at 60- or 90-day intervals*

Grasses	Number of analyses	February	March	April	May	June	August	September	October	November	December
From the college ranch plots in southern New Mexico:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Vine-mesquite	32	0.29	.....	0.55	.....	1.02	1.49	.....	1.02	.....	0.31
Alkali sacaton	32	.48	.....	.68	.....	.97	1.81	.....	1.45	.....	.67
Side-oats grama	32	.27	.....	.31	.....	.53	1.35	.....	.97	.....	.23
Saltgrass	22	.50	.....	.....	2.10	.....	1.03	.....	.....	1.11	.....
Bush muhly	21	1.34	.....	.....	1.40	.....	1.34	.....	.....	1.34	.....
Chamiza	22	2.71	.....	.....	3.62	.....	4.93	.....	.....	3.56	.....
Sand sagebrush	22	1.02	.....	.....	1.14	.....	2.42	.....	.....	1.61	.....
From the Estancia plots in central New Mexico:											
Blue grama	16	.....	0.30	.....	.....	1.16	.....	1.44	.....	.....	.41
Slender wheatgrass	16	.....	.49	.....	.....	2.38	.....	2.08	.....	.....	.63
Crested wheatrgass	16	.....	1.52	.....	.....	2.02	.....	2.01	.....	.....	.73
Smooth bromegrass	16	.....	3.13	.....	.....	2.26	.....	2.20	.....	.....	.86
Saltgrass	16	.....	.49	.....	.....	2.58	.....	1.71	.....	.....	.65
Alkali sacaton	16	.....	.50	.....	.....	3.02	.....	2.29	.....	.....	.78
Chamiza	15	.....	3.05	.....	.....	4.51	.....	3.33	.....	.....	1.33

Chamiso, an evergreen browse plant, has a high percentage of nonprotein. Results from Estancia in central New Mexico showed that the percentages of nonprotein for chamiza were lowest in December. Since winter temperatures of 10° to 20° below zero are common in this area, it is possible that during the winter this plant has much less activity in central New Mexico than in the southern part of the State. Sand sagebrush is another evergreen browse plant with pronounced growth during the growing season but with much less activity during the rest of the year. Crested wheatgrass and smooth bromegrass have significant high nonprotein values from March through September. Field observations also confirm the fact that these two grasses are very early starting in the spring. This is one of the reasons why they are being used for pasture reseeding purposes at some of the higher altitudes.

The botanist and plant breeder may make use of this nonprotein as an aid to field and other observations, if, when planning a project, the albuminoid nitrogen as well as the total nitrogen is to be determined upon the plant material.

SUMMARY

The percentages of nonprotein in various New Mexico range forages are presented. Some of the range grasses and browse plants have

continuous plant activity throughout the winter, though the rate of activity in certain species is much reduced. The percentages of nonprotein for black grama and mesa dropseed grasses are closely correlated with the carotene level. Analyses of the nonprotein, as well as of the crude protein and carotene values when these were available, together with field observations, formed the basis for estimating the plant activity of these forages.

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ALTERNARIA BLIGHT AND SEED INFECTION, A CAUSE OF LOW GERMINATION IN CERTAIN RADISH SEED CROPS[1]

By D. M. McLEAN

Research assistant, Section of Botany and Plant Pathology, Michigan Agricultural Experiment Station

INTRODUCTION

The production of radish seed is an important agricultural specialty in certain areas in Michigan. Seed crops usually are grown by farmers on a contract basis for various seedsmen and harvested seed must meet a minimum germination standard of about 85 percent. Seed crops which show substandard germination as specified by the seedman's tests frequently are discarded with a loss to the growers.

Seed crops of substandard germination generally occur in seasons of excessive rainfall subsequent to harvest, and it is during such seasons that radish plants usually are seriously blighted by infections of the *Alternaria* fungus. The disease is present in a greater or lesser degree every year.

In 1944 a project was initiated at the Michigan Agricultural Experiment Station to study the causes of low germination of radish seed crops. In a series of experiments Barrons and McLean[2] showed that the occurrence of low-germinating radish seeds may be influenced by high moisture in the storage bag after threshing. The growth of parasitic and saprophytic organisms was not correlated with high moisture during storage. In another experiment with samples of seeds from low-germinating lots they found approximately 40 percent infection by species of *Alternaria*. Four species of *Alternaria* were isolated from infected seeds harvested from a single planting.

Further studies were made in the field and in the laboratory during the season of 1945 to correlate pod infections by *Alternaria* with seed infections as a cause of low germination. This article records the results.

SYMPTOMS OF THE DISEASE

Since several species of *Alternaria* have been isolated from radish seeds (*Raphanus sativus* L.) taken directly from diseased pods, it is assumed that each organism must have invaded the seed through

[1] Received for publication May 22, 1946. Journal article No. 820 (n.s.) from the Michigan Agricultural Experiment Station.

[2] BARRONS, K. C., and McLEAN, D. M. A STUDY OF THE CAUSES OF LOW GERMINATION OF RADISH SEED CROPS. Mich. Agr. Expt. Sta. Quart. Bul 27: 398–408. 1945.

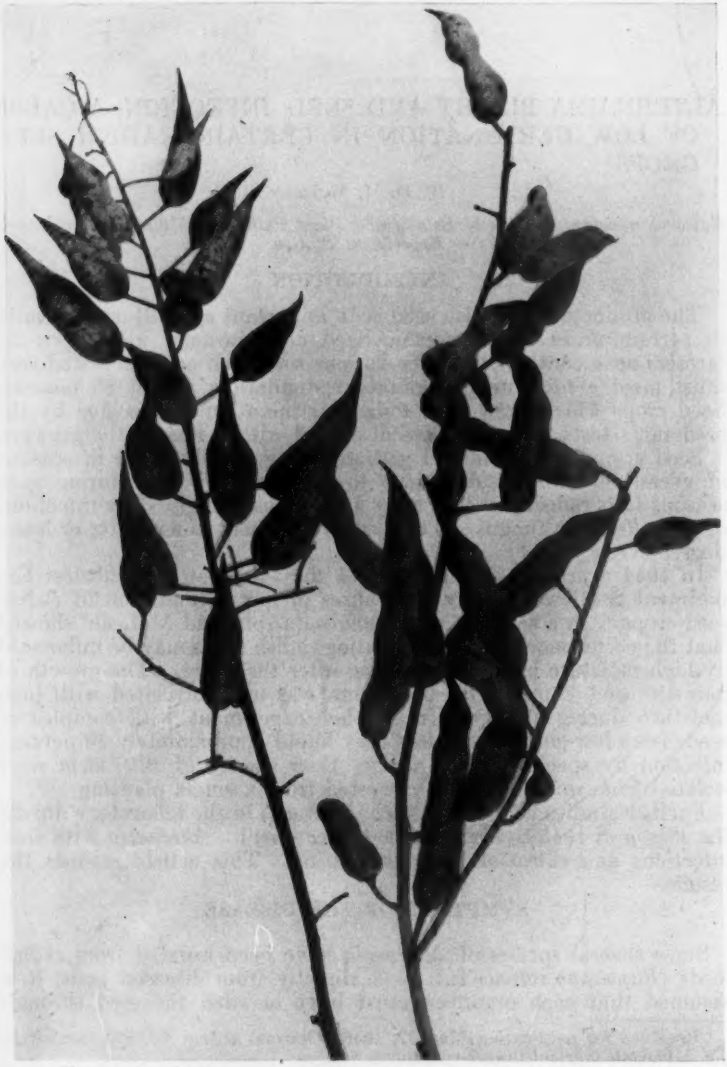

FIGURE 1.—Lesions on radish seed pods caused by *Alternaria raphani*.

the pods. No attempt has yet been made to determine the symptoms of the infections caused by each species. From a great number of isolations made from leaf, stem, pod, and seed infections *Alternaria raphani* Groves and Skolko has been observed. The symptoms, as they are described here, are those caused by infections of this fungus in inoculation experiments.

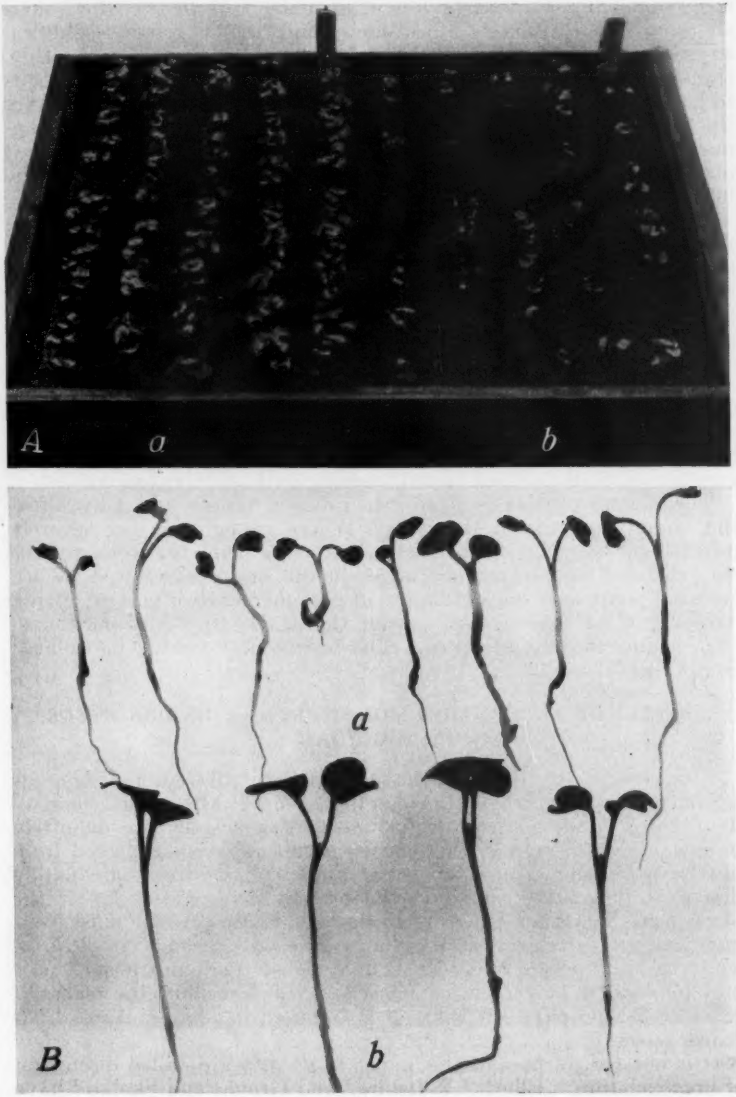

FIGURE 2. *A*, Seedbed showing effect of *Alternaria* infection on germination of radish seeds; *a*, seeds taken from apparently healthy pods; *b*, seeds taken from diseased pods. *B*, Radish seedlings: *a*, Infected by *A. raphani; b*, healthy.

Infections first become evident on the leaves of the fruiting stalk and may be found from the basal leaves to the tip leaves. The lesions first appear as very small yellowish-colored, slightly raised

spots one-half millimeter or less in diameter. With age, the lesions enlarge to approximately a centimeter and become roughly spherical to broaden elliptical. The centers of the older spots are of a thin, translucent, papery consistency usually accompanied by an olivaceous to grayish or blackish growth of the fruiting fungus. On older leaves the centers of the lesions may drop out leaving a "shot-holed" appearance. Individual lesions usually are bordered by a slightly raised margin or ridge. The fungus generally sporulates abundantly on the foliar lesions.

Before the seeds are mature, early pod and stem infections are visible as purplish to brownish irregular lesions (fig. 1). During periods of abundant rainfall, infections are so severe that entire pods may be included in the lesion. Usually, the stylar end of the infected pods is blackish and shriveled. When mature, the infected pods are often covered with blackish lesions from pin-point size to a centimeter or more (figs. 1 and 4, *B*, *a*). Only rarely are spores found on the pod lesions; however, some pods may be covered with a mass of olivaceous to blackish-colored spores. The fungus may be readily isolated from pod infections. Stem infections are common and the lesions resemble those on the pods (fig. 1). Infections may occur on exposed parts of the root crown, causing dark circular lesions.

The fungus penetrates deep into the pod tissues and may infect the immature seeds in the pods. Where young pods are severely infected the seeds may not develop. In older pods, the seeds may be shriveled and show darkened areas on the seed coats (fig. 4, *B*, *a*). Infected seeds may not germinate or pre-emergence or postemergence damping-off of seedlings may occur (fig. 2, *A*, *B*). Any portion of the seedling may be attacked. The fungus may readily be isolated from diseased seedlings.

SPECIES OF ALTERNARIA ISOLATED FROM RADISH SEEDS IN MICHIGAN

Four species of *Alternaria* have been isolated from radish seeds taken from crops grown in Michigan (fig. 3, *A–D*). Groves and Skolko [3] determined seven species of *Alternaria*, of which six are definitely pathogenic, from a great number of agricultural seeds collected from nearly world-wide sources. These writers have very adequately described the species found in the present investigation [3]. Of the four species found infecting radish seeds *A. raphani* is the most common and is extremely pathogenic. *A. brassicae* and *A. oleracea*, which are pathogenic to seedlings of various cruciferous hosts,[3] have been observed in only a few instances. *A. tenuis* includes the generally considered saprophytic forms and is common in plate cultures from radish seeds.

It is not the purpose of this paper to go into a detailed discussion of nomenclature. Elliott,[4] Wiltshire,[5] and Groves and Skolko [3] have

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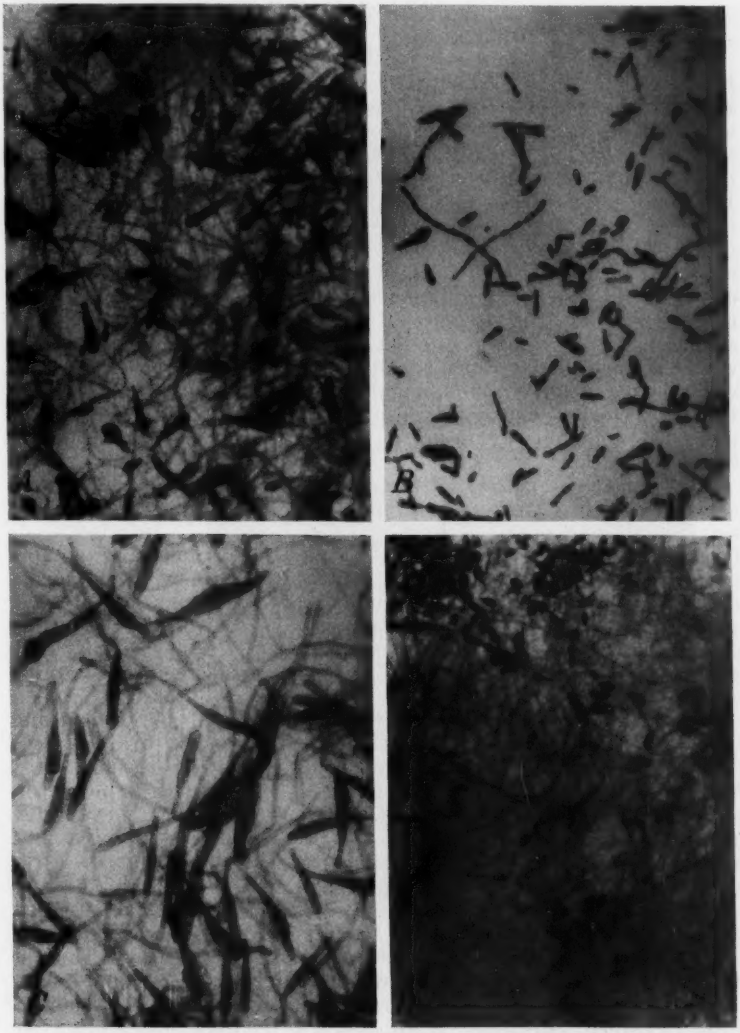

FIGURE 3.—Photomicrographs of spores of four species of *Alternaria* from radish seeds: *A, A. raphani; B, A. oleracea; C, A. brassicae; D, A. tenuis.*

thoroughly discussed the genus *Alternaria*. The identification of the species found in this investigation has been verified by Dr. J. W. Groves.[6]

[6] The writer acknowledges his indebtedness to Dr. Groves, of the Central Experimental Farm, Department of Agriculture Ottawa, Canada, for the verification of the species here discussed.

EXPERIMENTAL METHODS AND RESULTS OBTAINED

In a preliminary series of experiments conducted in 1944, it was found that a relatively high percentage of seeds of low-germinating seed lots were infected by parasitic and saprophytic organisms. Seed samples from a number of radish crops showing germination of 40 to 80 percent were obtained from seedsmen and growers in the State. Seeds from each sample were surface-sterilized by dipping them in 1 : 1,000 corrosive sublimate for 10 minutes after which they were rinsed in sterile water and placed on a potato-dextrose-agar medium in Petri plates. After several days, the *Alternaria* fungus was growing from as many as 40 percent of the seeds in certain samples. The counts of infected seeds were made without attempting to determine the relative number of species. *Fusarium*, *Penicillium*, *Rhizopus*, and certain bacteria were present in occasional instances, but these were not considered a principal cause of low germination. *A. raphani* was the organism most commonly found on seed samples of low vitality. Groves and Skolko also found species of *Alternaria* to be the most common organisms on low-germinating radish seeds in Ontario, Canada.

Seed treatment with the common dust materials did not significantly increase germination in the low-germinating samples. The same result was obtained by Groves and Skolko.[7] Infected seeds treated in a water bath at 50° C. (122° F.) for 25 minutes germinated free of *Alternaria* on agar media. Hot water treatment, however, did not increase germination percentages in low-germinating diseased seeds. It seemed evident that whatever the cause of low germination it was operative before the seeds were marketed or even before they were harvested. With this point in mind, further experimentation was conducted in 1945.

Diseased plants with mature pods were collected in the field and brought to the laboratory, where they were thoroughly dried. Seeds from individual diseased pods showing numerous lesions and seeds from individual pods apparently free from infection were placed on potato-dextrose agar in Petri plates in the same position that they occupied in the pods. These seeds were not surface-sterilized, but were transferred directly from the pods to the Petri plates. After several days, seeds from pods with numerous lesions showed 75 to 80 percent infection with *Alternaria*. Often all the seeds in the diseased pods were infected (fig. 4, *B*, *b*). Usually, the seeds directly beneath the pod lesions were infected and frequently the seed coat showed dark markings. Seeds from pods which had only an occasional lesion, the majority of which appeared healthy, likewise frequently showed 10 to 15 percent infection. *A. raphani* was the only fungus observed in this experiment.

In another experiment, seeds were taken from diseased pods and from apparently healthy pods for testing germination (fig. 4, *A*, *a* and *b*). Seeds taken from diseased pods were slightly smaller than those from apparently healthy pods and many showed dark markings on the seed coat. Seeds which were shriveled and those which proba-

[7] See footnote 3, p. 74.

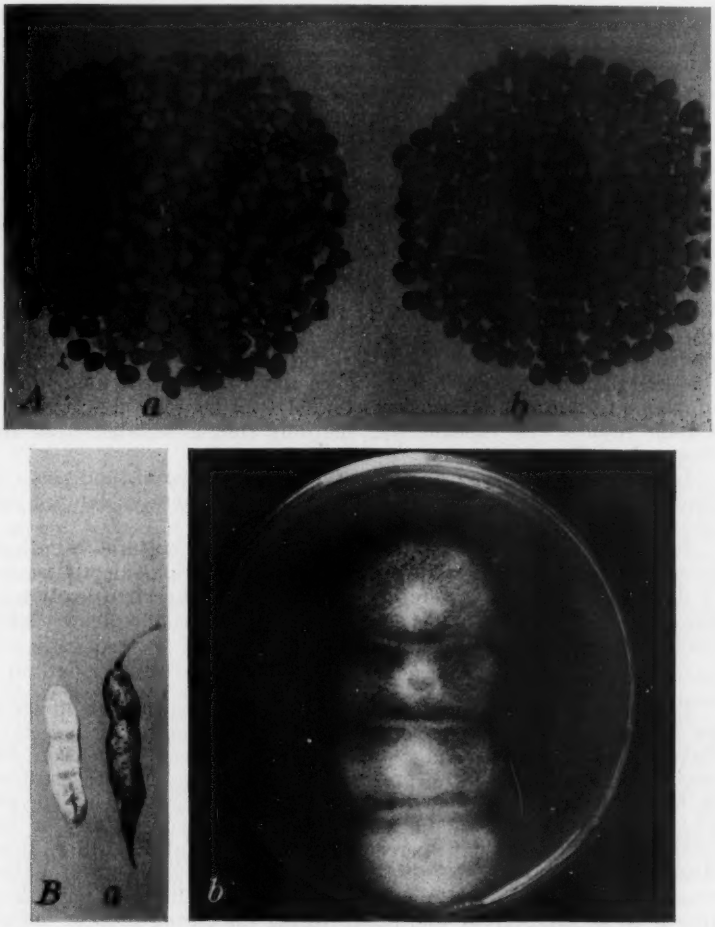

FIGURE 4.—*A, a,* Radish seeds taken from pods infected with *Alternaria,* showing dark areas on seed coats; *b,* seeds taken from apparently healthy pods. *B, a,* Diseased radish pods; *b, A, raphani* growing on seeds taken from diseased pods and placed on potato-dextrose agar.

bly would be eliminated in the cleaning process after threshing were removed from the lot. Samples of 500 seeds taken from diseased pods and from apparently healthy pods were planted in sterilized sand. After 9 days the number of seedlings were counted in each case. The results are presented in table 1.

TABLE 1.—*Germination percentages of radish seeds taken from diseased pods and from apparently healthy pods*

Block No.	Seeds from diseased pods		Seedlings infected	Seeds from apparently healthy pods		
	Planted	Germinated		Planted	Germinated	Seedlings infected
	Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
1	100	36	9	100	90	13
2	200	27	10	200	87	14
3	200	35	11.5	200	81	14.5
4	500	31	.....	500	85	.....
5	500	32	.....	500	85	.....

The data in table 1 show (1) that *Alternaria* infection causes a marked reduction in germination of seeds harvested from diseased pods, and (2) that seed taken from apparently healthy pods in a diseased planting may be infected even though there is little evidence of pod infection. The experiment was repeated several times with similar results. *A. raphani* was the only fungus isolated from the diseased seedlings.

Lesions were observed on the cotyledons, hypocotyl, and roots of diseased seedlings, and infected seedlings frequently damped off (fig. 2 *B*, *a*).

It was during the course of this experiment that cultural variations were noticed in the different *Alternaria* isolates from infected seeds. A detailed study of the different isolates led to the identification of the species previously mentioned. *A raphani* was the species most commonly observed.

DISCUSSION

The results of the foregoing experiments have shown that infection by *Alternaria* is a principal cause of low germination in radish seed of certain crops. Groves and Skolko [8] found a direct correlation between germination and the presence of *A. raphani* in seed samples in which germination ranged from 14 to 65 percent. This species was the only pathogenic fungus that appeared consistently in all their samples in a high percentage. The organisms other than *Alternaria* which are frequently isolated from radish seeds are probably unimportant as a cause of low germination. For the most part they appear to be saprophytic and occur during the curing process before the seeds are dry. High moisture during storage may cause increased infections by the *Alternaria* fungus. When pod infections occur, the spores are mixed with the seed during threshing, and the mycelium may continue to infect the seeds and give a moldy appearance to the crop.

Since it has been shown that percentage germination of seeds from diseased pods is lower than that of seeds from apparently healthy pods, it appears that low germination may be correlated directly with pod infections in the field. This seems to be indicated also by the periodicity of good and bad seed crops in Michigan. Radish plantings for seed crops are blighted with *Alternaria* infections nearly

[8] See footnote 3, p. 74.

every year in Michigan. In certain seasons crops are harvested which give high germination percentages, while in others germination is low. Low germination percentages are usually found in seasons of excessive rainfall when *Alternaria* infection is serious. Various reasons have been given to account for low germination during these years, including (1) poor pollination, (2) wet weather during the curing period, (3) excessive moisture after sacking from the threshing machine, and (4) the prevalence of seed-borne pathogens. Some of these reasons are correlated with conditions which favor infection by *Alternaria*.

Since neither fungicidal seed treatments nor hot water disinfection of diseased seeds necessarily induces a higher germination of infected seed, control measures must be applied in the field. Inasmuch as *Alternaria raphani* seems to be the principal cause of the disease and apparently has been isolated only from radish, it follows that if disease-free seed were planted the severity of seed infection might be controlled by destruction of the source of primary inoculum.

SUMMARY

Four species of *Alternaria* have been isolated from low-germinating radish seeds in Michigan. *A. raphani* causes infections of the leaves, stems, pods, and roots of radish plants and is the most common parasitic species that has been isolated. Seeds taken from infected pods frequently show 70 to 80 percent infection. This species is also pathogenic to seedlings. *A. raphani* is considered to be the cause of infection in the field which results in low germination in infected seed crops. *A. brassicae* and *A. oleracea* are found only rarely and probably are not of primary importance as a cause of low germination in seed crops. *A. tenuis* is a type commonly found on radish seeds, but it is considered to be mostly saprophytic. *Penicillium*, *Rhizopus*, *Fusarium*, and certain bacteria frequently are found in seeds after the curing process.

Fungicidal dust treatments of low-germinating seeds have failed to give significant increases in percentage of seedling counts. Hot water treatment for 25 minutes at 50° C. (122° F.) killed the *Alternaria* pathogen in infected seeds. Such treatment, however, did not increase germination precentages in low-germinating seed lots.

U. S. GOVERNMENT PRINTING OFFICE: 1947